^{1}H-Magnetic Resonance Spectroscopy for Quantifying Myocardial Lipid Content in Humans With the Cardiometabolic Syndrome

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Elevated serum free fatty acid (FFA) levels are common in people at risk for cardiometabolic syndrome. These FFA are transported, converted, and stored as monoglycerides, diglycerides, and triglycerides in nonadipose tissues including liver, skeletal muscle, and heart. For the heart, this lipid accumulation has been associated with insulin resistance, diabetes, systolic dysfunction, and in animal models, apoptosis.[1–7] However, the relationship between FFA, cardiometabolic syndrome, and cardiovascular disease is unclear and the correlations imperfect. In contrast to FFA levels, a direct measure of cardiac intramyocellular lipid (IMCL) content provides a clear indicator of ectopic lipid accumulation and may provide a better risk predictor for disease. Here we describe and demonstrate a ^{1}H magnetic resonance spectroscopy (MRS) technique for quantifying human myocardial lipid content.

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Manuscript received August 11, 2008; revised March 4, 2009; accepted April 3, 2009

doi: 10.1111/j.1559-4572.2009.00061.x

PROTOCOL

The MRS protocol for measuring myocardial IMCL is based on a free-breathing, point-resolved spectroscopy (PRESS) sequence with alternating gradient polarity every average, optimized crushers, COG5(2,3,2:0) phase cycling, and both electrocardiographic (ECG) and 2-dimensional prospective acquisition correction respiratory gating.[8–11] As no water suppression is used, the alternating gradient polarity minimizes spectral distortions and generally provides more robust quantification; the optimized crushers minimize the echo time (Te) while ensuring suppression of outer volume signal from pulse imperfections; and, considering motion, the reduced 5 step (10 with alternating gradient polarity) phase cycling similarly minimizes outer volume signal by reducing the cycle time. The respiratory gating occurs at end expiration and timed via a 2-dimensional navigator with a 2 mm window. The navigator is placed on the diaphragm as far from the heart as reasonable using a set of rapid repetition, free-breathing coronal images. Voxel location in the interventricular septum is then determined from similarly ECG and navigator gated, short and long axis true fast imaging with steady state precession

(true-FISP) images. The delay after the R-wave ECG trigger for the PRESS sequence to begin (typically 150–170 milliseconds), is determined from one sequence of end-expiration breath-hold cine images. After the voxel location and trigger delay have been determined, the transmitter power and frequency are optimized and an automatic shim is performed on a 50% enlarged voxel; the voxel is enlarged because these procedures are not gated. Ten PRESS averages are then collected and Fourier transformed to verify that the H_2O full-width half-height line width is below approximately 0.3 ppm.[10]

Optimally, the main PRESS acquisition proceeds by collecting blocks of 60 averages at both a short and long Te point, followed by one of the navigator-gated true-FISP images to verify that the patient has not moved. If only the relative change of IMCL is needed, for example to gauge an intervention, only the short Te point is needed. For the data presented below, the imaging system was a Siemens (Erlangen, Germany) 1.5T MAGNETOM Sonata, with the body coil used as the transmitter and an 8-element chest array coil for reception. The specific acquisition parameters that were used are: Te_1 and Te_2 set to 24 and 60 milliseconds; the repetition time (Tr) equal to multiples of the respiratory cycle, generally at least 3.5 seconds; and, 120 averages collected per Te (2 blocks) with the receiver bandwidth set to 2 kHz, 512 acquisition points, and a voxel size of 20×20×6 mm.

For the protocol, the data are processed and analyzed using the software jMRUI 3.0 and AMARES, respectively.[12,13] The processing consists of a 5 Hz Gaussian apodization, zero filling to 2048 points, and both manual frequency shifting and zero order phasing, typically both of which are minimal. Similar Te blocks are then added together. The AMARES fitting routine is employed in 2 stages, with the goal of modeling the IMCL resonances after the H_2O line shape. To this end, the H_2O resonance (4.7 ppm) is fit with Lorenzian and Gaussian apodized sinusoids constrained to the same frequency and an additional Lorenzian sinusoid. After this initial fit, the IMCL methylene resonance (1.3 ppm) is fit with similarly constrained sinusoids, ie in relation to the corresponding H_2O sinusoids, the amplitude ratios and relative frequency shift are equivalent; the widths are 20% wider; and, only the overall frequency and amplitude of the methylene resonance are varied. A fourth Lorenzian sinusoid with a "soft" width constraint of 5–20 Hz is also included for the methylene resonance. The IMCL methyl (0.9 ppm) and 2.1 ppm resonances are modeled similarly with 4 sinusoids each except that their respective sinusoids are scaled by 0.134 and 0.21 to those of the corresponding methylene sinusoids, and only the overall frequencies of these resonances are varied.[14] Other fitting criteria include: all phases fixed to zero; non-fixed frequencies constrained to ±0.2 ppm of the values stated; and, 512 AMARES points with the first point truncated.

IMCL content is typically presented as the ratio of the area of the IMCL resonances to that of the H_2O resonance.[3,7] However, this ratio must be corrected for T_2 relaxation so that it is not dependent on the experimental Te values. With 2 Te data sets, the relaxation corrected ratio, S_0, is[10,15]:

$$S_0 = \frac{\mathrm{IMCL}_A}{\mathrm{H_2O}_A} \left(\frac{\mathrm{IMCL}_A \cdot \mathrm{H_2O}_B}{\mathrm{IMCL}_B \cdot \mathrm{H_2O_A}}\right)^{\frac{\mathrm{Te}_A}{\mathrm{Te}_B - \mathrm{Te}_A}} \quad (1)$$

wherethe subscripts A and B reference a value at a particular Te; IMCL and H_2O refer to the AMARES amplitudes of the respective resonances; and, complete longitudinal relaxation is assumed (long Tr). In the protocol, the IMCL resonances refer to those of the methyl and methylene (0.7–1.7 ppm) nuclei and not those adjacent to a carbonyl or double bond moiety (≈2.0–2.4 ppm).[16] If a single Te value is used, S_0 is calculated using literature relaxation values:

$$S_0 = \frac{\mathrm{IMCL}_A}{\mathrm{H_2O}_A} \cdot \mathrm{Exp} \times [\mathrm{Te}_A(1/\mathrm{T}_{2,\mathrm{IMCL}} - 1/\mathrm{T}_{2,\mathrm{H2O}})] \quad (2)$$

where$T_{2,IMCL}$ and $T_{2,H2O}$ are the IMCL and H_2O T_2 values and are 86 and 40 milliseconds, respectively.[17] For completeness, a unit conversion formula can be found in the literature.[6,17,18] For these formula, the average triglyceride molecule has a molecular weight of 855 g/mol with 75 ^1H nuclei resonating between 0.7 and 1.7 ppm.[14]

The study participants (Table) were 2 men infected with human immunodeficiency virus (HIV). These patients were receiving highly active antiretroviral therapy and had significant risk factors for the cardiometabolic syndrome (central adiposity, insulin resistance, dyslipidemia).[1] These studies were approved by the Human Research Protection Office at Washington University School of

Table. Patient Characteristics

Characteristic	Patient A	Patient B
Age, y	43	40
Current HIV medications	Ritonavir, truvada, atazanavir	Combivir, efavirenz, atripla
Height, cm	185	170
Weight, kg	116	110.45
BMI, kg/m^2	33.7	38.14
Waist, cm	123	117
Body fat, %	29.7	33.4
Trunk-to-limb fat ratio	1.39	1.49
Glucose, mg/dL	89	102
Insulin, μU/mL	25	14.3
HOMA-IR	5.3	3.6
Total cholesterol, mg/dL	237	209
LDL cholesterol, mg/dL	164	120
HDL cholesterol, mg/dL	40	49
Triglycerides, mg/dL	167	202
Systolic/ diastolic BP, mm Hg	136/85	135/74

Abbreviations: BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; HOMA-IR, homeostasis model assessment for insulin resistance; LDL, low-density lipoprotein. All hormone, metabolite, and blood pressure measurements were made after an overnight fast.

Medicine, and participants signed an approved informed consent document.

RESULTS

The Figure shows the voxel location and the resulting ^{1}H-spectrum obtained on patient A. The insets on the right show the scaled IMCL regions of both patients and their percent IMCL relative to H_2O, corrected for relaxation using Eq. 2. The coefficients of variation (CV) for patients A and B (Te=24 milliseconds, 3 measurements) were 6.4% and 4.1%, respectively. Based on 34 repeated measurements from 11 research patients with a range of body mass indices from 18 to 33 kg/m^2, the above protocol has an overall CV of approximately 9%, which is consistent with approximately 5% to 17% reported by others.[3,15,17–19] It should be noted that the 34 measurements had IMCL signal-to-noise ratios above 5 and H_2O half height full width line widths <0.3 ppm. These criteria are necessary for the described processing to achieve this CV.

DISCUSSION

This ^{1}H-MRS approach for quantifying myocardial lipid content is noninvasive, requires approximately 40 minutes of scanner time, is applicable to any modern (≥1.5T) magnetic resonance imaging instrument,

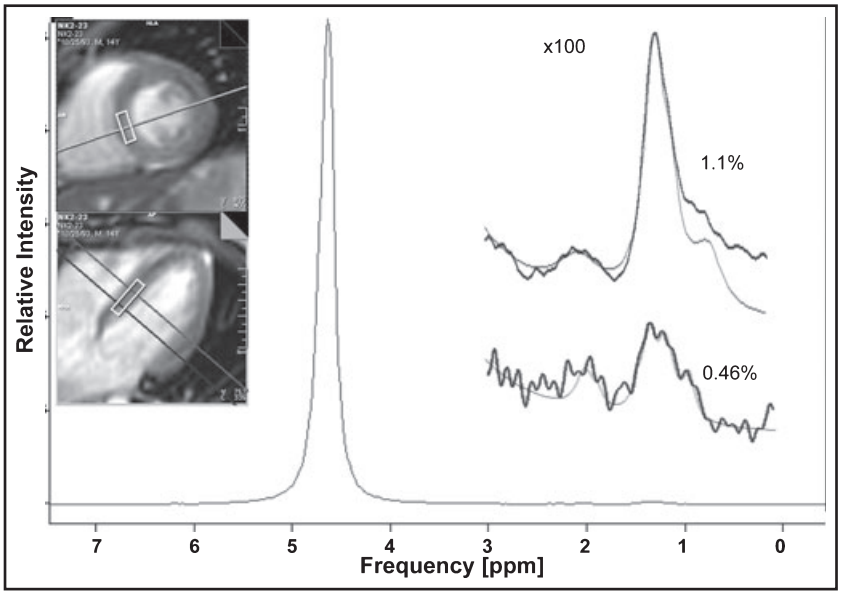

Figure. ^{1}H magnetic resonance spectroscopy spectrum at 1.5T from the interventricular septum of patient A with echo time=24 milliseconds and 120 averages. The upper left images show the voxel location. The resonances at 4.7 and 1.3 ppm are from H_2O and the intramyocellular lipid (IMCL) methylene hydrogen nuclei, respectively. The lower insert on the right is from the spectrum itself, scaled up 100×, and the upper right insert is the scaled equivalent from patient B. The percentages represent the IMCL area relative to the H_2O area from the AMARES fit (smooth, lighter line).

and has an acceptable CV and specificity.[20] With a CV of 9%, approximately 12 patients would be required to detect a 10% change in IMCL, and n=40 for a 5% change (based on α=0.05; β=0.1 or power=0.9).[21] The main potential contaminant concerning specificity is extramyocellular lipid (EMCL) from pericardial fat, which resonates between 1.2 and 1.6 ppm, depending on its orientation.[6,15] The assay relies on a small, well localized voxel in the interventricular septum to minimize the pericardial lipid signal and assumes the remaining signal is from lipid within myocardial cells, or IMCL. At the expense of lower signal-to-noise ratio, this localization essentially eliminates EMCL signal, and allows for a simpler, more robust processing scheme. Other potential contaminants include methyl and methylene resonances from other species, which are minimal, and additional H_2O signal from blood, which is also minimal because the PRESS sequence is insensitive to species undergoing macroscopic motion.[11]

Generally, MRS is not considered a standard clinical procedure, partially because of perceived difficulties in the data analysis.[22] With minimal interference from pericardial EMCL that results from the localization in the interventricular septum, the analysis of this protocol is comparatively simple and straightforward, especially after the spectra have been weighted and phased. After these initial steps, fitting templates are loaded into jMRUI (*freeware*), altered a bit for reference values and ratios, and run. In future versions of jMRUI, more complicated constraints, user plug-ins, etc, may completely automate the analysis. Still, overall the procedure is simpler and less time consuming than the ex vivo and, consequently, invasive biochemical assay for triglycerides.[23] However, unlike the biochemical analysis, which measures glycerol and has a one to one mole correspondence with triglycerides, the MRS assay measures the ratio of triglyceride to H_2O hydrogen nuclei. Thus, if absolute units are required, average tissue water content and triglyceride molecular weights values must be used and accuracy is reduced. Similar reductions occur when average relaxation values are used instead of the 2-Te point protocol.[10] However, a single Te protocol is probably quite adequate for measuring intrasubject IMCL change during an intervention.

Perhaps the most important aspect of this assay can be seen by examining the relative amounts of myocardial lipid found for patients A and B, Figure. Although both have cardiometabolic syndrome, the percent IMCL for patient A (0.46%) is typical of lean people. Based on other studies and the 2-fold higher percent IMCL for patient B (1.1%), one might predict that patient B has a higher risk of developing type II diabetes and/or cardiovascular disease.[7] Additional studies may establish such a predictive value for this technique.

CONCLUSIONS

We describe and characterize a ^{1}H-MRS technique for quantifying human myocardial lipid content, apply it to 2 HIV-infected men with several risk factors for cardiometabolic syndrome, and quantify markedly different levels in the 2 patients. With further use and testing, this measure may be validated for assessing the risks associated with myocardial steatosis and the effects that myocardial lipid content have on insulin signaling, apoptosis, and the pathogenesis of myocardial dysfunction. With regard to clinical application, this straightforward, accurate assay may provide valuable diagnostic and prognostic information and a noninvasive, in vivo method to evaluate interventions for metabolic syndrome, diabetes, dyslipidemia, and cardiovascular disease in general.

Acknowledgments and disclosure: We thank the patients for their participation. These studies were supported by NIH grants P20 RR020643, DK049393-12, DK059531-08, DK074343-03, UL1-RR024992, and DK056341-08.

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